Bulletin of Environment, Pharmacology and Life Sciences
Bull. Env. Pharmacol. Life Sci., Spl Issue [2] 2023: 074-078

Online ISSN 2277-1808
Journal's URL:http://www.bepls.com
CODEN: BEPLAD

ORIGINAL ARTICLE **OPEN ACCESS**

Microbial Study of Homemade Paneer Sold in Sangli Market

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ABSTRACT

Paneer is an important Indian traditional coagulated dairy product that provides nutrition with variety of flavors, texture to the consumers while productivity and profitability to the mostly small-scale traders and halwa is which produces this dairy product by traditional methods. The unhygienic condition maintained during manufacturing leads to the lower shelf life of the paneer. As the demand for paneer is increasing every year, there is a great concern to produce high quality with long life products that requires hygienic modern processing with preservation technologies. The organized dairies in India have to modernize and scale up the production in order to meet the demand. Paneer is an acid coagulated dairy product, which is similar to western cottage cheese and Tofu (soya panner). In India, paneer production has been largely confined to small non organized sectors. The microflora comes from cooling water used for immersing, environmental contaminations, unhygienic conditions, storage temperature, etc. This results in post manufacturing changes in fat and protein and loss moisture during storage. In this paper we have studied microbial flora of homemade paneer sold in the market of Sangli, Maharashtra, India.

Key words: *Paneer, Microflora, Unhygienic conditions*

Received 28.05.2023 Revised 20.08.2023 Accepted 23.09. 2023

INTRODUCTION

The milk being a complete food, has been recognized as an important diet [1]. About half the milk produced is consumed in the liquid form and the remaining is used to prepare products such as *ghee*, curd, *butter*, *khoa*, *paneer*, cheese, *chhana*, ice cream and milk powders [2]. *Paneer* is obtained by heat treating the milk following acid coagulation using citric acid, lactic acid, tartaric acid, alum, sour whey. The whey is removed by filtration and pressing. *Paneer* belong to soft varieties of cheese family and is used in culinary dishes. About 5% of milk produced in India is converted into *paneer* [3].Quality of paneer depends upon type of milk, fat percentage, type of coagulant, heat treatment of milk, moisture content and yield of paneer etc.The spoilage of paneer is majorly due to microbial action [4].Microbial contamination can be a potential hazard during pre and post processing leading to food poisoning [5].The microbiological analysis of paneer is highly recommended from food quality point of view. So, the aim of the present work was to assess the quality of homemade paneer from Sangli.

MATERIAL AND METHODS

Collection of Paneer Sample

A total 10 homemade paneer samples were purchased randomly from different local shops of various sectors in Sangli. Samples were collected in pre-sterilized container and transported in ice-bucket to the laboratory and immediately processed for further work (Table-1).

Isolation and Identification of Microbial Isolates from Paneer Samples

Serial dilutions were prepared of each paneer sample in Ringer's solution and 0.1ml from each dilution was spread inoculated on various laboratory media like Nutrient Agar, Mac-Conkey's agar, Selenite F Broth, Deoxycholate citrate Agar, Blood Agar, Sabouraud's Agar, YEMA Agar. All the plates were incubated at room temperature for 24-48 hrs. After incubation well isolated colonies from each plate were selected and identified on the basis of colony characters and suspensions were prepared for further identification. Isolates were obtained and designated as Isolate 1, Isolate 2, Isolate 3, Isolate 4 and Isolate 5.

Preservation of Isolates-Preservation of isolates was done on agar slant containing the media which was used for isolation. The slants were preserved in refrigeration temp at 4°C and sub cultured after 20days.

Characterization of the isolates

These microbial isolates were subjected for identification on the basis of morphological, cultural and biochemical characters. The isolates were identified by using Bergey's manual of systemic bacteriology Volume 1 and Volume 2.

a) **Cultural and morphological characteristics:** The obtained isolates were studied for their colony characteristics & morphological characteristics Gram staining was performed by Hucker and Cohn method and motility was observed by hanging drop method.

b) **Biochemical characteristics of isolates:**

Utilization of carbohydrates: Utilization of carbohydrates was studied using the basal medium of peptone water and 1% of the carbohydrate as Glucose, Fructose, Mannose, Lactose, Maltose, Sucrose, Mannitol and Sorbital.

Enzymatic Activities of The Isolates: The various enzymatic activities of the isolates were studied as tests below-

a) **Catalase Test** was performed by picking the colony with the help of sterile nichrome wire loop & immersing the wire loop in 10% hydrogen peroxide solution in the test tube. Production of oxygen as small bubbles indicates positive test and if not, then catalase test is negative

b) **Citrate Utilization Test**: A loop ful suspension of each isolate was aseptically inoculated on the Koser's citrate slant. A slant without inoculation was used as control. Growth on slant indicates test as positive while no growth indicates test as negative.

c) **Nitrate Reduction Test:** Nitrate reduction test is indicated by inoculating loop ful of suspension of each bacterium into peptone nitrate broth tube and incubated at 28°C for 24 hrs. After incubation the tube was observed for growth. After incubation alpha napthyl amine and sulfanilic acid are added and presence of nitrite was studied by formation of red colour.

d) **Starch Hydrolysis:** This test is indicated by cross streaking a loopful of suspension of each bacterium onto starch agar plate. After inoculation the plates were incubated at 28^0C for 24 hrs. After incubation the plates was treated with Iodine and the clear zone of starch hydrolysis surrounding the growth was said to be positive.

e) **Sugar Fermentation Test:** Tubes containing respective sugar and peptone water were inoculated with loopful suspension of each bacterial isolate and incubated at 28 °C for 24 hrs. After incubation tubes were observed for acid and gas formation. Various sugars tested were dextrose, mannitol, fructose, sucrose, lactose and Arabinose.

d) **Indole Test:** For the production of indole, loopful suspension of each bacterial isolates were inoculated into a sterile peptone broth tube. This tube was incubated at 28°C for 24 hrs. After incubation the production of indole was detected by use of Kovacs reagent.

e) **Methyl Red Test**: Loopful suspensions of bacterial isolates were inoculated into a sterile glucose phosphate broth tube and it was incubated at 28°C for 24 hrs. The utilization of glucose and production of acid was detected by developing of pink color after addition of methyl red indicator.

b) **Voges-Proskaur Test**: Loopful suspensions of bacterial isolates were inoculated into a sterile glucose phosphate broth tube and it was incubated at 28°C for 24 hrs. In this test production of acetyl methyl carbinol from glucose is detected by using 40% KOH and a-naphthol solution.

MBRT TEST: MBRT TEST as described by Harrigen (1976)

1) 1 gm of Homemade Paneer sample was taken in a clean glass test tube and the slurry was made in sterile distilled water.

2) Sample was mixed thoroughly.

3) 10 ml sample was taken in sterile glass tube and 1ml of the Methylene Blue (1:250000 diluted) was added under aseptic condition.

Positive control was prepared by adding 1ml sterile distilled water to 10 ml sample.

4) Negative control was prepared by boiling paneer sample in sterile tube and then addition of 1 ml Methylene Blue was done.

5) All the tubes were plugged with cotton swab and inverted once or twice and placedin the water bath at 37 °C.

6) The tubes were observed after every half an hour interval until the blue colour from the last test tube has disappeared (not more than 8 hours).

7) Quality of homemade paneer sample was determined on the basis of the time required for the reduction of Methylene blue.

Most Probable Number (MPN) Test on Paneer

Presumptive Test:

In this standard method 15 fermentation tubes each containing 10ml of sterile Mac-conkey's broth were used five tubes containing double strength sterile Mac-conkey's broth and 10 tubes containing single strength Mac-conkey's broth.
They were inoculated with 5x 10 ml, 5x 1ml, 5 x 0.1ml of home made paneer sample and then incubated at 37°C for 24-48 hours and examined for acid and gas production. Production of acid and gas was taken as presumptive evidence of coliform. The MPN of coliforms was determined from the number of tubes which showed acid and gas production by referring McCardy's table.

Confirmed Test:

A loopful from the tubes showing gas production was streak inoculated on Eosin Methylene Blue agar plates and plates were incubated at 35°C for 24 hours and examined for typical colonies of *E. coli* with green metallic sheen. When typical colonies were seen the completed test was carried out.

Completed Test:

Colony from EMB agar plates was sub cultured into lactose broth fermentation tube and nutrient agar slant. Both were incubated at 37°C for 24 hours: Gas production in broth Gram negative non-spore forming rod from the agar slant was taken evidence of coliforms. In order to determine the type of coliforms, the isolates were subjected to IMViC.

Standard Plate Count (SPC):

Serial dilutions of homemade paneer sample were prepared using sterile dilution blanks 0.1 ml of each dilution was inoculated on sterile Nutrient agar plates. The plates were incubated at 37°C for 24 to 48 hours. After incubation colonies were counted and result were expressed as **SPC= No. of colonies x Dilution factor 10**.

RESULTS AND DISCUSSION

10 homemade paneer samples were purchased from differ local shops of various local shops in Sangli. A total of 5- microbial isolates were obtained from these paneer samples were designed as PS1, PS2, PS3, PS4 and PS5.
Colony characteristics of isolates on the Nutrient agar at 28^0C for 48-h(Table-2).
Isolates PS1,PS4 and PS5 were found to be 1mm in size while PS2 was 2mm and PS3 was 3mm in size. Isolates PS2 and PS3 were milky in colour. Isolates PS1 was yellow and isolate PS4 was cream and PS5 was white in colour. Isolates PS1, PS2, PS3 and PS4 are entire in margin and PS5 having undulate margin. Isolates PS1, PS2, PS3 and PS4 were convex elevation and PS5 was raised. PS1, PS2, PS3 and PS4 were sticky and PS5 was dry. All the isolates were opaque.
On the basis of morphological, cultural and biochemical characteristics five isolates i.e. PS1,PS2,PS3,PS4 and PS5 were tentatively identified as: *Staphylococcus aureus, Escherichia coli, Bacillus spp., Streptococcus spp. and Lactobacillus spp.*
MBRT was given positive by sample no. 2, 4, 7 and 9. Thus these samples show poor quality while rests are all good quality. MPN count was high in sample no. 2, 4, 7 and 9. Thus it shows a poor quality of paneer with respect to coliforms... SPC was also found to be more in by sample no. 2, 4, 7 and 9. Out of total 10 paneer samoles,2, 4, 7 and 9 were of poor and reject quality.
On the basis of morphological, cultural and biochemical characteristics five isolates were tentatively identified as (Table-5)

Details of Collection of Paneer Sample

Sr. No.	Date	Place of collection	Type of paneer	Amount of paneer
1	8/11/2022	Ganesh dudhdairy, Rammandir corner, Sangli.	Home made	100 gm
2	9/11/2022	Khandagaledudh dairy, Haripur, Sangli.	Home made	100 gm
3	10/11/2022	Dadgaedugdhalya, Shavajiputala, Sangli	Home made	100 gm
4	11/11/2022	Aditya dairy, Patel chowk,Sangli	Home made	100 gm
5	12/11/2022	Gokuldudh dairy, Miraj road, Sangli	Home made	100 gm
6	13/11/2022	Madusudhan dairy, Maruti chowk, Sangli	Home made	100 gm
7	14/11/2022	Ramvishwasdudh Dairy, Gauligali, Sangli	Home made	100 gm
8	15/11/2022	Shivprasaddudhdairy.Ganesh Nagar. Sangli	Home made	100 gm
9	16/11/2022	Krishana dairy, near Udagavkarhospital,Sangli	Home made	100 gm
10	17/11/2022	Gouri dairy, Vishram bag, Sangli	Home made	100 gm

Quality of Homemade Paneer Sample on The Basis of MBRT Time Was Done as Below

Quality of Home-Made Paneer Sample	Decolorization Time in MBRT
Excellent	More than 8 h
Good	Between 6-8 h
Fair	Between 2-6 h
Poor	Less than 2 h

Table 1: Morphological Characters of Isolate PS1, PS2, PS3, PS4 and PS5.

Paneer Isolate no	Morphology	Gram Nature	Motility
PS1	Cocci	Gram positive	Non motile
PS2	Short rods	Gram negative	Motile
PS3	Long rods	Gram positive	Motile
PS4	Cocci	Gram positive	Non motile
PS5	Straight rods	Gram positive	Motile

Table-2: Colony Characters of Isolates PS1, PS2, PS3,PS4 and PS5.

Paneer Isolate no	Colony Characters of isolates						
	Size	Shape	Colour	Margin	Elevation	Opacity	Consistency
PS1	1mm	Circular	Yellow	Entire	Convex	Opaque	Miost
PS2	2mm	Circular	Milky	Entire	Convex	Opaque	Miost
PS3	3mm	Circular	Milky	Entire	Convex	Opaque	Miost
PS4	1mm	Circular	Cream	Entire	Convex	Opaque	Miost
PS5	1mm	Circular	White	Undulate	Raised	Opaque	Dry

Table 3: Biochemical Characteristics of Isolates of PS1, PS2, PS3, PS4 and PS5.

Biochemical tests	PS1	*PS2*	*PS3*	*PS4*	*PS5*
Sugar tests					
Glucose	+	+	+	-	-
Arabinose	-	-	-	-	-
Xylose	-	+	-	-	-
Sucrose	+	-	-	-	-
Maltose	+	+	-	-	-
Mannitol	+	-	-	-	-
Lactose	-	+	-	-	-
Galactose	+	-	-	-	+
Sorbitol	-	-	-	-	-
Fructose	+	-	-	-	-
Ribose	+	-	-	+	+
NaCl tests					
3%	-	-	-	-	-
4%	-	-	+	-	-
6.5%	-	-	+	+	-
10%	+	-	+	+	+
15%	+	-	-	-	-
pH 9.6	-	-	-	+	-
Growth on 40% Bile agar	-	-	-	+	-
Growth at 40^{0}C	-	-	+	+	-
IMViC tests					
Indol	-	+	-	-	-
Methyl red	-	+	-	-	-
Vogues proskaur	-	-	-	-	-
Citrate	-	-	-	-	-
Catalase	-	+	-	-	-
Starch	-	+	-	-	-
Strictly aerobic	+	-	+	+	-
Strictly anaerobic	-	+	-	-	+
Novobiosin resistance	-	-	-	-	-
Nitrate reduction	+	+	-	-	-

+ :- Indicates positive test - :- Indicates negative test

Table 4 Results of MPN, MBRT and SPC of Paneer Samples

Samples No.	SPC PFU/g	MBRT test	MPN Index/ 100mL
1	$236x10^5$	-	<6
2	$148 x10^5$	+	>2400
3	$40 x10^5$	-	21
4	$228 x10^6$	+	>2400
5	$49 x10^5$	-	9
6	$888 x10^9$	-	26
7	$184 x10^6$	+	350
8	$22 x10^3$	-	17
9	$64 x10^5$	+	540
10	$237 x10^6$	-	<6

+ :- Indicates positive test - :- Indicates negative test

Table 5: Identification of Isolates

Sr. No.	Paneer isolate no.	Tentative identification of isolates
1	PS1	*Staphylococcus aureus*
2	PS2	*Escherichia coli*
3	PS3	*Bacillus spp.*
4	PS4	*Streptococcus spp.*
5	PS5	*Lactobacillus spp.*

CONCLUSION

Some pathogenic microorganisms were isolated from various homemade paneer samples and identified by performing various biochemical tests SPC, MBRT and MPN was also performed on each homemade paneer sample.

Following conclusions could be drawn from the work:

Isolates PS1,PS2,PS3,PS4 and PS5 was tentatively identified as *Staphylococcus spp., Escherchia spp. Bacillus spp., Streptococcusspp.* and *Lactobacillus spp.* isolated from some homemade pancer samples.

The bacteriological quality of these homemade paneer samples was also not much good.

MPN and SPC was also found to be high for these homemade paneer samples.

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CITATION OF THIS ARTICLE

Jadhav A. V, Patil Y. M., Walekar S. S. and Pathade G. R., Microbial Study Of Homemade Paneer Sold In Sangli Market. Bull. Env. Pharmacol. Life Sci., Spl Issue [2]: 2023: 074-078.